CLAIM LISTING

Claim 1 (Currently Amended): A method of monitoring expression of two or more genes in one or more cells, comprising:

obtaining a population of RNA from a sample comprising fewer than 1000 cells;

generating a first population of cDNA from the RNA; [[and]]

linearly amplifying the first population of cDNA to produce [[an]] a linearly amplified second population of cDNA;

labeling the linearly amplified second population of cDNA with a detectable label to generate a linearly amplified population of labeled cDNA;

contacting an array of probes with the linearly amplified population of labeled cDNA; [[and]]

determining relative hybridization of the probes to the linearly amplified population of labeled cDNA; and

determining relative expression of at least two genes.

Claim 2 (Previously Presented): The method of claim 1, wherein the population of RNA is derived from a single cell.

Claim 3 (Previously Presented): The method of claim 1, wherein the first population of cDNA is prepared by reverse transcription of a population of mRNA.

Claim 4 (Original): The method of claim 3, wherein the reverse transcription is conducted under conditions of incomplete extension.

Claim 5 (Previously Presented): The method of claim 1, wherein the population of RNA is derived from fewer than 100 cells.

Claim 6 (Original): The method of claim 1, wherein the conditions of incomplete extension are effected by use of limited reagents, by use of a shorter time than required for complete extension, by use of a suboptimal temperature, or by incorporation of a chain-terminating nucleotide.

Claim 7 (Original): The method of claim 1, wherein the conditions of incomplete extension synthesize polynucleotides with a median length of about 100-1000 bases.

Claim 8 (Original): The method of claim 1, wherein the conditions of incomplete extension synthesize polynucleotides with a median length of about 500-700 bases.

Claim 9 (Original): The method of claim 1, wherein the genes to be monitored are of at least partly known sequence, and the probe array comprises a probe set for each gene to be monitored, the probe set comprising a plurality of probes perfectly complementary to or perfectly matched to a transcript.

Claim 10 (Original): The method of claim 9, wherein at least some of the probes in each probe set are perfectly complementary to or perfectly matched to a segment within 1000 bases from the 3' end of the sequence of the transcript.

Claim 11 (Original): The method of claim 9, wherein at least one probe in each probe set is perfectly complementary to or perfectly matched to a segment within 1000 bases from the 3' end of the coding sequence of the transcript.

Claim 12 (Original): The method of claim 9, wherein the probes in the probe set are perfectly complementary to or perfectly matched to one gene, one gene family or one gene cluster in the plurality of transcripts to be monitored.

Claim 13 (Original): The method of claim 9, wherein at least ten probes in each probe set are perfectly complementary to or perfectly matched to a segment within 500 bases from the 3' end of the coding sequence of the transcript.

Claim 14 (Original) The method of claim 9, wherein the probe array comprises a probe set for at least 1000 genes.

Claim 15 (Original): The method of claim 9, wherein the probe array comprises a probe set for at least 10,000 genes.

Claim 16 (Original): The method of claim 9, wherein each probe set further comprises a mismatch probe for each perfectly matched probe, the mismatched probe differing from the perfectly matched probe at a single position.

Claim 17 (Original): The method of claim 9, further comprising comparing the hybridization of matched and mismatched probes to determine the relative expression levels of the genes.

Claim 18 (Original): The method of claim 9, wherein the relative expression levels of each of 1000 genes are determined.

Claim 19 (Original): The method of claim 9, wherein the relative expression levels of each of 10,000 genes are determined.

Claim 20 (Previously Presented): The method of claim 9, further including the step of determining relative expression levels of detected genes and wherein the relative expression levels of detected genes vary by at least about 2-fold to about ten-fold.

Claim 21 (Previously Presented): The method of claim 9, further including the step of determining relative expression levels of detected genes and wherein the relative expression levels of detected genes vary by at least 100 fold.

Claim 22 (Previously Presented): The method of claim 9, further including the step of determining relative expression levels of detected genes and wherein the relative expression levels of detected genes vary by at least 1000 fold.

Claim 23 (Previously Presented): The method of claim 1, wherein the probe array comprises all probes of a given length.

Claim 24 (Original): The method of claim 1, wherein at least some of the plurality of genes are of unknown sequence.

Claim 25 (Previously Presented): The method of claim 2, further comprising cleaving nucleic acids into fragments.

Claim 26 (Original): The method of claim 25, further comprising end-labeling the fragments.

Claim 27 (Original): The method of claim 1, wherein the array comprises at least 1000 probes per cm^2.

Claim 28 (Original): The method of claim 1, wherein the probes are nucleic acids of 15-25 bases.

Claim 29 (Original): The method of claim 1, wherein the probes are attached to a nonporous support.

Claim 30 (Original): The method of claim 1, wherein the one or more cells are obtained from a biopsy without in vitro propagation of the cells.

Claim 31 (Original): The method of claim 20, wherein the one or more cells are obtained from a tissue known or suspected to be neoplastic.

Claim 32 (Currently Amended): A method of expression monitoring comprising,

obtaining a first population of RNA from a first cell;

obtaining a second population of RNA from a second cell;

generating a population of first cell cDNA from the first population of RNA and linearly amplifying the population of first cell cDNA to produce [[an]] a linearly amplified population of first cell cDNA;

generating a population of second cell cDNA from the second population of RNA and linearly amplifying the population of second cell cDNA to produce [[an]] a linearly amplified population of second cell cDNA;

labeling the linearly amplified populations of first cell and second cell cDNA with a detectable label to generate a linearly amplified first population and a linearly amplified second population of labeled cDNA;

contacting an array of probes with the linearly amplified first and second populations of labeled cDNA; and

determining the relative binding of the probes to the linearly amplified first and second populations of labeled cDNA to identify at least two probes binding to at least two genes that are differentially expressed between the first and second cells.

Claim 33 (Previously Presented): The method of claim 32, wherein the first population and the second population of labeled cDNA are differentially labeled and simultaneously applied to the array of probes.

Claim 34 (Previously Presented): The method of claim 32, wherein the first population and the second population of labeled cDNA are applied separately to the array of probes.

Claim 35 (Original): The method of claim 32, wherein the array of probes comprises a plurality of probes perfectly complementary to or perfectly matched to each of a plurality of known transcripts.

Claim 36 (Original): The method of claim 32, further comprising using a probe that binds to a differentially expressed gene to clone the gene.

Claim 37 (Original): The method of claim 32, further comprising searching a database for a nucleic acid sequence including a sequence from a probe that hybridizes to a differentially expressed gene.

Claim 38 (Original): The method of claim 32, wherein the first and second cells are at different stages of development within a common cell lineage.

Claim 39 (Currently Amended): A method of classifying cells, comprising:

obtaining a single cell population of RNA from each cell of a plurality of single cells;

generating a first population of cDNA from the RNA and linearly amplifying the first population to produce [[an]] a linearly amplified second population of cDNA;

labeling the linearly amplified second population of cDNA with a detectable label to generate a linearly amplified population of labeled cDNA;

determining an expression profile of each of the single cells by contacting an array of probes with the linearly amplified population of labeled cDNA, determining relative hybridization of the probes to the linearly amplified population of labeled cDNA, and determining relative expression of at least two genes; and

classifying the single cells in clusters determined by similarity of expression profile.

Claim 40. (Currently Amended): A method of monitoring differentiation of a cell lineage, comprising:

obtaining a single cell population of RNA from each cell of a plurality of single cells at different differentiation stages within the lineage;

generating a first population of cDNA from the RNA and linearly amplifying the first population to produce [[an]] a linearly amplified second population of cDNA;

labeling the linearly amplified second population of cDNA with a detectable label to generate a linearly amplified population of labeled cDNA;

determining an expression profile of each of the single cells by contacting an array of probes with the linearly amplified population of labeled cDNA, determining relative hybridization of the probes to the linearly amplified population of labeled cDNA, and determining relative expression of a plurality of genes;

classifying the single cells in clusters determined by similarity of expression profile;

ordering the clusters by similarity of expression profile; and

determining a time course of expression levels for each of the genes at different stages of differentiation in the cell lineage.

Claim 41 (Original): The method of claim 1, wherein the conditions of incomplete extension synthesize polynucleotides with a median length of about 20-100 bases.

Claim 42 (Currently Amended): A method for identifying differentially expressed transcripts, comprising:

obtaining a single cell population of RNA from each cell of a plurality of single cells at different differentiation stages within a lineage;

generating a first population of cDNA from the RNA and linearly amplifying the first population to produce [[an]] a linearly amplified second population of cDNA;

labeling the linearly amplified second population of cDNA with a detectable label to generate a linearly amplified population of labeled cDNA;

determining an expression profile of a plurality of genes of each of the single cells by contacting an array of probes with the linearly amplified population of labeled cDNA, determining relative hybridization of the probes to the linearly amplified population of labeled cDNA, and determining relative expression of a plurality genes;

classifying the single cells in clusters determined by similarity of expression profile;

ordering the clusters by similarity of expression profile;

determining a time course of expression levels for each of the plurality of genes at different stages of differentiation in the cell lineage; and

identifying differentially expressed transcripts.

Claim 43 (Currently Amended): A method of identifying a specific cell type comprising:

obtaining a single cell population of RNA from each cell of a plurality of single cells;

generating a first population of cDNA from the RNA and linearly amplifying the first population to produce [[an]] a linearly amplified second population of cDNA;

labeling the linearly amplified second population of cDNA with a detectable label to generate a linearly amplified population of labeled cDNA;

determining an expression profile of each of the single cells by contacting an array of probes with the linearly amplified population of labeled cDNA, determining relative hybridization of the probes to the linearly amplified population of labeled cDNA, and determining relative expression of a plurality genes;

classifying the single cells in clusters determined by similarity of expression profile;

determining the nature and function of a plurality of single cells.

Claim 44 (Previously Presented): The method of claim 42 wherein the plurality of cells originates from the adult brain.

Claim 45 (Previously Presented): The method of claim 41 wherein the plurality of cells originates from peripheral sensory organs.

Claim 46 (Previously Presented): The method of claim 41 wherein one single cell from the plurality of cells can be deduced to have stem cell potentials.